TECHNIQUES FOR OBTAINING ADULT-ASSOCIATED IMMATURE STAGES OF PREDACIOUS TACHYDROMIINE FLIES (DIPTERA: EMPIDOIDEA), WITH IMPLICATIONS FOR REARING AND BIOCONTROL[1]

J. M. Cumming, B. E. Cooper[2]

ABSTRACT: Gravid females of four species of tachydromiine flies, namely *Megagrapha exquisita, Platypalpus holosericus, P. aequalis,* and *P. melleus* were induced to oviposit by decapitation. Eggs were placed on a saline nutrient agar medium prior to hatching. Ovaries containing fully mature eggs were additionally dissected from females of *P. holosericus.* These eggs were transferred *in situ* in each ovary to agar plates, where they embryonated and hatched, demonstrating parthenogenesis in this species. First instar larvae of all four species were held in agar medium for several weeks, and were presented with various prey organisms and other food materials. Only those that fed on *Drosophila melanogaster* larvae, or on each other, developed to later larval instars. In one instance, a fully mature larva of *M. exquisita* pupated after diapausing, and developed as far as the teneral adult stage. Implications of the results of this study are discussed in terms of the potential for obtaining taxonomic and phylogenetic information on previously unknown immature stages, and for rearing Tachydromiinae as biological control agents of agricultural pests.

The beneficial nature of empidoid flies as predators of insect pests has long been recognized (reviewed for example by Smith, 1969, p. 18), with the potential economic importance of one subfamily, the Tachydromiinae, recently attracting considerable interest[3]. For example, adult tachydromiines have been identified as important regulators of small Diptera, Thysanoptera and aphid pests in cereal and oil seed crops (Berest, 1987; Brunel *et al.,* 1989; Chvála, 1975; Crook and Sunderland, 1984; Jones, 1965, 1969, 1976a, 1976b; Potts and Vickerman, 1974; Stark, 1990; Stark and Wetzel, 1987; Sunderland *et al.,* 1985), leafmining flies in greenhouse and field situations (Kovalev, 1966; Rotheray, 1989; Whitfield, 1925), as well as psyllids and phytophagous mites in orchards (Chvála, 1975; Fleschner and Ricker, 1953).

Despite this interest, effective use of tachydromiines as biological control agents of agricultural pests has been severely hindered by a lack

[1] Received July 3, 1992. Accepted September 26, 1992.

[2] Biological Resources Division, Centre for Land and Biological Resources Research, Agriculture Canada, Research Branch, Central Experimental Farm, Ottawa, Ontario, Canada K1A 0C6.

[3] Classification of the Tachydromiinae within either the Empididae or Hybotidae is discussed in Cumming and Cooper (1992).

of information about immature stages and life histories of empidoid flies in general. For example, no empidid species has ever been successfully reared through all life stages in the laboratory. Even the informative outline of the life cycle of *Empis tessellata* Fabricius, described by Hobby and Smith (1961), was pieced together from collections of mature larvae taken from leaf litter (reared to adults) and from eggs obtained from mated females (hatched to first instar larvae). The entire literature on the structure and habits of immature stages of the Tachydromiinae, essentially amounts to brief descriptions of the larva of a *Platypalpus* species by Beling (1888), the larva and pupa of a *Drapetis* species by Malloch (1917), and the larva of *Crossopalpus curvipes* (Meigen) by Smith (1989). Smith (1989) additionally lists rearing records of adults of *Crossopalpus* sp. from dung, *C. nigritellus* (zetterstedt) from fungi, *Elaphropeza ephippiata* (Fallén) from woodland soil, and *Tachypeza nubila* (Meigen) from fungi and under bark, indicating that the immatures of Tachydromiinae appear to occupy various terrestrial habitats.

The primary purpose of this paper is to add to our knowledge of the Tachydromiinae by reporting on techniques used to obtain adult-associated immature stages and on preliminary investigations into the establishment of a rearing method for members of the subfamily. in addition, a procedure is described for determining female reproductive mode.

PROCEDURE AND RESULTS

Rearing Method. Adult females of *megagrapha exquisita* (Malloch), *Platypalpus holosericus* Melander, *P. aequalis* Loew, and *P. melleus* Melander were netted locally during the summer months and placed individually in plastic tubes containing moistened tissue paper, for transport back to the laboratory[4]. Gravid females with fully mature eggs were induced to oviposit by decapitation, following the method described by Linley (1965) for the ceratopogonid fly species *Leptoconops bequaerti* (Kieffer). This was most easily achieved after slightly anaesthetizing a specimen with CO_2 and transferring it to moistened filter paper before removing the head with fine dissecting scissors. Decapitated females generally started ovipositing immediately and sometimes continued to lay eggs for up to an hour after oviposition commenced.

Following oviposition, eggs from each female were transferred with a fine brush to the surface of a saline nutrient agar medium that had been allowed to set to a depth of approximately 4 mm in a 50 X 9 mm seal-tight

[4] Voucher specimens are deposited in the Canadian National Collection of Insects and Arachnids (CNC).

petri dish (Fig. 1). The saline nutrient agar medium used to rear specimens of Tachydromiinae was originally developed for rearing predacious larvae of the ceratopogonid fly species *Culicoides melleus* (Coquillett) and is fully described by Linley (1985). The advantages discussed by Kettle *et al.* (1975) in using agar to rear *Culcoides* larvae, and the necessity demonstrated by Linley (1985) of adding supplementary vitamins for rearing predacious larvae such as *Culicoides* (see below under "Food Requirements"), appear generally applicable to the rearing procedure employed here. Seal-tight petri dishes, which are designed with tight-fitting lids (Fig. 1), are a necessary modification, essential in containing the small highly motile tachydromiine larvae in the agar medium.

The agar-filled petri dishes were maintained in the dark, in an environmental chamber at a constant 20° C. Darkening of the chorion of the egg on the first day denoted initiation of embryogenesis in all four species, and indicated that the eggs were either fertilized during oviposition, or were developing parthenogenetically. In all four species, larval cephalic structures and segmentation usually became visible through the semi-transparent chorion towards the end of the first week after oviposition (Fig. 2), with the eggs generally hatching by the end of the second week. In some eggs of *P. holosericus,* hatching was delayed up to at least four weeks after oviposition without noticeable developmental effects, by keeping the eggs at 11° C.

First instar larvae of all four species appeared to burrow through the agar medium easily (Figs. 3-4), and could be kept alive in the tightly sealed petri dishes with little maintainence for several weeks. Larvae seemed unaffected by fungus and bacteria, even when some older cultures became heavily contaminated with these microorganisms, and often appeared somewhat attracted to these contaminants (Fig. 6). Those that fed (see below under "Food Requirements") appeared to progress through three larval instars, as described for the distantly related empidoid, *Liancalus virens* Scopoli (Vaillant, 1948). The larval growth rate for all species varied considerably as experimentation to determine food requirements progressed, although in one batch of eggs of *M. exquisita,* final instar larvae (Fig. 5) developed relatively rapidly, within approximately five weeks from the time of hatching. Final instar larvae of all species that were still alive towards the end of the summer were cooled down and allowed to diapause at 1° C for three months. Most larvae survived the diapause period, and in one instance, a single larva of *M. exquisita* pupated (without forming a cocoon) in the agar medium approximately five weeks after the temperature was increased to 15° C. Within three weeks the pupa developed to the teneral adult stage (Fig. 8),

but died before eclosion occurred.

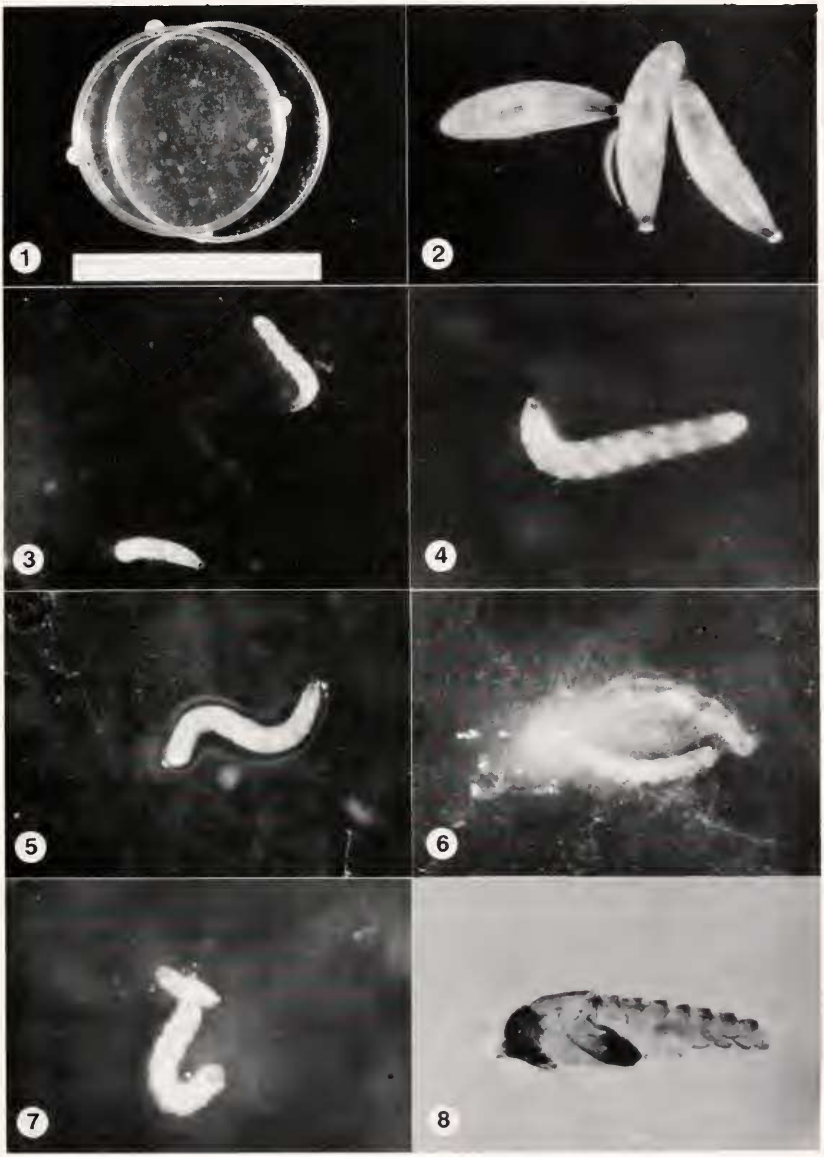
Food Requirements. Attempts were made to determine the food requirements of the larvae, since the diet of larval tachydromiines is unknown. Although never directly established, tachydromiine larvae have been assumed to be predacious (Chvála and Kovalev, 1989), based on limited observations of other empidoid species (reviewed by Smith, 1969, p. 6). First instar larvae of all four tachydromiine species were offered various small soil organisms, such as nematodes belonging to the genus *Panagrellus,* all stages of the oribatid mite species *Oppia nitens* C.L. Koch, and an inoculum of protozoans, but feeding on these microorganisms was not observed. In addition, early as well as later instar larvae did not appear to scavenge on dead organisms, or on moistened pieces of highly proteinaceous dried puppy meal, which were also added to some cultures.

Finally, small larvae of the pomace fly, *Drosophila melanogaster* Meigen, were presented as prey. Larvae of all four species of tachydromiines readily fed on the *Drosophila* larvae within the agar medium (Fig. 7). The smaller first instar tachydromiine larvae however, were only able to overpower the smallest (first instar) *Drosophila* larvae. Later tachydromiine instars fed on *Drosophila* larvae of various sizes, and occasionally these older predacious larvae also cannibalized smaller sibling larvae in the same culture. Since *Drosophila* larvae could survive in the agar medium for one or two days before starving, periodic replacement of prey larvae was required to sustain the tachydromiine larval cultures.

Reproductive Mode. Females of *P. holosericus* are suspected of reproducing parthogenetically (as has been suggested by Tuomikoski, 1935 and Chvála, 1975 for some Palearctic species of *Platypalpus*), since males of this common Nearctic species are not represented in the main North American empidoid collections, and have never been collected locally. To determine the reproductive mode in *P. holosericus,* ovaries were dissected from four gravid females and transferred individually to eight agar plates for observation. During dissection each ovary, containing an average of approximately 30 fully mature eggs, was surgically removed from the lateral oviduct to prevent any possibility of accidental fertilization. Despite heavy fungal contamination of the ovariole tissue, the occurrence of parthenogenesis in *P. holosericus* was convincingly demonstrated when most of the eggs in all eight of the dissected ovaries hatched.

DISCUSSION

The results obtained to date provide a first step in the development of a general procedure for rearing tachydromiine flies, which will aid in the accumulation of valuable taxonomic and life history data. Most impor-



Figs. 1-8. 1, Seal-tight petri dish filled with saline nutrient agar medium for rearing Tachydromiinae (0.6 X); 2, eggs of *Platypalpus holosericus* Melander containing developing larvae (33 X); 3, first instar larvae of *P. holosericus* in agar medium (18 X); 4, first instar larva of *P. holosericus* in agar medium (36 X); 5, late instar larva of *Megagrapha exquisita* (Malloch) on top of agar medium (12 X); 6, first instar larvae of *P. holosericus* amongst fungal contamination of agar medium (27 X); 7, late instar larva of *P. holosericus* feeding on early instar larva of *Drosophila melanogaster* Meigen (18 X); 8, lateral view of pupa of *M. exquisita* containing teneral adult (14 X).

tantly, induction of oviposition behavior by decapitation, in conjunction with maintenance of the eggs in agar-filled petri dishes to avoid desiccation, appears to be a useful technique for obtaining adult-associated first instar larvae (and possibly later stages) of Tachydromiinae[5]. The technique may work with, and should be attempted on, other poorly known Empidoidea (e.g. Atelestinae, Brachystomatinae, Ceratomerinae, Microphorinae, and *Nemidina*) and taxonomically problematic Cyclorrhapha (e.g. Opetidae), for which immature stages are not known (see for example Sinclair, 1992). Even if the first instar larvae obtained are not reared successfully to a further stage, the taxonomic information gained from having properly associated immatures of any stage for such groups, would be valuable for testing previously proposed classifications and formulating new phylogenetic hypotheses.

Dissection of ovaries from parthenogenetic species, such as *P. holosericus*, can also yield large numbers of first instar larvae. Of perhaps greater significance however, is the use of this dissection procedure for determining whether certain females within a species, or all females, are able to reproduce parthenogenetically rather than bisexually. Parthenogenesis is presumed to occur in certain species of the genus *Platypalpus*, where males have been rarely collected, or remain unknown. In the Palearctic Region, some species are thought to be entirely parthenogenetic [e.g. *P. major* (Zetterstedt) (Chvála, 1975, 1989)], or partially parthenogenetic [e.g. *P. ecalceatus* (Zetterstedt) (Chvála, 1989; Tuomikoski, 1935)] throughout their range, whereas others [e.g. *P. candicans* (Fallén) and *P. cursitans* (Fabricius) (Chvála, 1975; Frey, 1943; Tuomikoski, 1935)] appear to exhibit geographic parthenogenesis. Conclusive determination of reproductive mode for species of *Platypalpus* at the population level could be important for future screening of possible biological control agents. This is because increased reproductive potential associated with parthenogenesis can be a desirable attribute for beneficial insects being considered for release programs (Aeschlimann, 1990; Doutt *et al.*, 1976), and mating requirements for parthenogenetic females can be effectively ignored.

Larval food requirements of empidoids in general are poorly understood, and no information is available for Tachydromiinae. The procedures outlined here however, allow for experimentation with various prey organisms and other food materials to determine which broad categories of food types can be consumed by larvae. The results

[5] Morphological study of the immature stages of the Tachydromiinae will be dealt with in subsequent papers.

obtained on the four species studied here, indicate that larvae of many if not all Tachydromiinae are predacious, probably on small soil or litter inhabiting organisms such as other Diptera larvae, rather than being saprophagous or microorganism feeders. The use of larval Diptera as the major source of prey for the larvae of some other empidoid groups, has been noted by Smith (1969). The apparent attraction of the larvae of all four tachydromiine species towards areas of heavy fungal and bacterial contamination in older cultures, suggests that natural organisms captured by these predacious larvae probably include small mycetophagous or saprophagous Diptera larvae, or other soft-bodied prey.

A general procedure for rearing tachydromiine flies could have important implications for biological control programs targeted against a variety of small-sized insect pests. For example, the predatory activity of many adult tachydromiines appears to be both intense and of long duration (Chvála, 1975; Stark and Wetzel, 1989; Whitfield, 1925), and adults are considered to occupy small-sized predator niches not generally shared by other (usually larger-sized) predators (Chvála, 1975). This, in conjunction with the ability of several species to reach very high population densities (e.g. recorded as high as 40 to 60 individuals of *Platypalpus* per meter^2 in cereal crops by Stark, 1990), and the apparent lack of a fixed diapause stage in at least some species of *Crossopalpus, Platypalpus,* and *Stilpon* (Chvála, 1975) suggests potential benefits for the development of future mass-rearing programs for this group of predacious flies. Common parthenogenetic species like the relatively large, voracious *P. holosericus* in North America, or the very similar European *P. major,* appear to be ideal candidates for further research.

ACKNOWLEDGMENTS

We would like to thank A. Borkent (Salmon Arm, B.C.) for his discussions on ceratopogonid rearing techniques and J. R. Linley (University of Florida, Vero Beach) for graciously supplying supplementary vitamins for the saline nutrient agar. J. E. O'Hara (Biological Resources Division—CLBRR) and B. J. Sinclair (Carleton University, Ottawa) kindly reviewed the manuscript and suggested many improvements.

LITERATURE CITED

Aeschlimann, J. P. 1990. Simultaneous occurrence of thelytoky and bisexuality in Hymenopteran species, and its implications for the biological control of pests. Entomophaga 35: 3-5.

Beling, T. 1888. Beitrag zur Metamorphose einiger zweiflügeliger Insecten aus den Familien Tabanidae, Empidae, und Syrphidae. Verh. Zool.—Bot. Ges. Wien 38: 1-4.

Berest, Z. L. 1987. Trophic relations of natural enemies of cereal leaf aphids. Vestnik Zool. 21: 45-48. [In Russian.]

Brunel, E., P. Grootaert and J. Mesquida. 1989. Entomofaune associee a la floraison du colza (*Brassica napus* L.): Note preliminaire sur les Dolichopodidae et les Empididae (Insectes: Diptera). Med. Fac. Landbouww. Rijksuniv. Gent 54(3a): 727-737.

Chvála, M. 1975. The Tachydromiinae (Dipt. Empididae) of Fennoscandia and Denmark. Fauna Entomol. Scand. 3: 1-336.

Chvála, M. 1989. Monograph of northern and central European species of *Platypalpus* (Diptera, Hybotidae), with data on the occurrence in Czechoslovokia. Acta Univ. Carol.—Biol. 32: 209-376.

Chvála, M. and V. G. Kovalev. 1989. Family Hybotidae, pp. 174-227. *In*: Soós, Á. and L. Papp (eds.), Catalogue of Palaearctic Diptera. Vol. 6. Therevidae—Empididae. Akadémiai Kiadó, Budapest. 435 pp.

Crook, N. E. and K. D. Sunderland. 1984. Detection of aphid remains in predatory insects and spiders by ELISA. Ann. Appl. Biol. 105: 413-422.

Cumming, J. M. and B. E. Cooper. 1992. A revision of the Nearctic species of the tachydromiine fly genus *Stilpon* Loew (Diptera: Empidoidea). Can. Entomol. 124: 951-998.

Doutt, R. L., D. P. Annecke and E. Tremblay. 1976. Biology and host relationships of parasitoids, pp. 143-168. *In:* Huffaker, C. B. and P. S. Messenger (eds.), Theory and Practice of Biological Control. Academic Press, New York. 788 pp.

Fleschner, C. A. and D. W. Ricker. 1953. An empidid fly predacious on citrus red mites. J. Econ. Entomol. 46: 155.

Frey, R. 1943. Übersicht der paläarktischen Arten der Gattung *Platypalpus* Macq. (= *Coryneta* Meig.). (Dipt. Empididae). Notul. Entomol. 23: 1-19.

Hobby, B. M. and K. G. V. Smith. 1961. The bionomics of *Empis tessellata* F. (Dipt., Empididae). Entomologist's Mon. Mag. 97: 2-10.

Jones, M. G. 1965. The effects of some insecticides on populations of frit fly (*Oscinella frit*) and its enemies. J. Appl. Ecol. 2: 391-401.

Jones, M. G. 1969. The effect of weather on frit fly (*Oscinella frit* L.) and its predators. J. Appl. Ecol. 6: 425-441.

Jones, M. G. 1976a. The arthropod fauna of a winter wheat field. J. Appl. Ecol. 13: 61-85.

Jones, M. G. 1976b. Arthropods from fallow land in a winter wheat-fallow sequence. J. Appl. Ecol. 13: 87-101.

Kettle, D. S., C. H. Wild and M. M. Elson. 1975. A new technique for rearing individual *Culicoides* larvae (Diptera: Ceratopogonidae). J. Med. Entomol. 12: 263-264.

Kovalev, V. G. 1966. On the fauna and ecology of predacious Diptera of the subfamily Tachydromiinae (Diptera, Empididae) in the central European belt of the USSR. Entomol. Review 45: 436-438.

Linley, J. R. 1965. The ovarian cycle and egg stage in *Leptoconops (Holoconops) becquaerti* (Kieff.) (Diptera, Ceratopogonidae). Bull. Entomol. Res. 56: 37-56.

Linley, J. R. 1985. Growth and survival of *Culicoides melleus* larvae (Diptera: Ceratopogonidae) on four prey organisms. J. Med. Entomol. 22: 178-189.

Malloch, J. R. 1917. A preliminary classification of Diptera, exclusive of Pupipara, based upon larval and pupal characters, with keys to imagines in certain families. Part 1. Bull. Illinois St. Lab. Nat. Hist. 12: 161-409, pls. 28-57.

Potts, G. R. and G. P. Vickerman. 1974. Studies on cereal ecosystems. Adv. Ecol. Res. 8: 107-197.

Rotheray, G. E. 1989. Prey capture in *Tachydromia annulipes* (Meigen) (Diptera: Empididae). Br. J. Entomol. Nat. Hist. 2: 177-179.

Sinclair, B. J. 1992. A phylogenetic interpretation of the Brachycera based on the larval mandible and associated mouthpart structures. Syst. Entomol. 17: 233-252.

Smith, K. G. V. 1969. The Empididae of southern Africa (Diptera). Ann. Natal Mus. 19: 1-347.

Smith, K. G. V. 1989. An introduction to the immature stages of Bitish flies. Diptera larvae, with notes on eggs, puparia and pupae. Handbk. Ident. Br. Insects 10 (Part 14): 1-280.

Stark, A. 1990. Flies of the genus *Platypalpus* (Hybotidae, Empidoidea) as predators of cereal pests. 2nd Int. Congr. Dipt., Bratislava. Abstr. Vol. p. 226.

Stark, A. and Th. Wetzel 1987. Fliegen der Gattung *Platypalpus* (Diptera, Empididae)—bisher wenig beachtete Prädatoren im Getreidebestand. J. Appl. Entomol. 103: 1-14.

Sunderland, K. D., R. J. Chambers, D. L. Stacey and N. E. Crook. 1985. Invertebrate polyphagous predators and cereal aphids. Bull. Int. Org. Biol. Contr. Noxious Animals & Plants SROP/WPRS 8(3): 105-114.

Tuomikoski, R. 1935. Ein vermullicher Fall von geographischer parthenogenesis bei der Gattung *Tachydromia* (Dipt., Empididae). Ann. Entomol. Fenn. 1: 38-43.

Vaillant, F. 1948. Les premiers stades de *Liancalus virens* Scop. (Dolichopodidae). Bull. Soc. Zool. France 73: 118-130.

Whitfield, F. G. S. 1925. The natural control of the leaf-miner *Phytomyza aconiti* Hendel (Diptera) by *Tachydromia minuta,* Meigen (Diptera). Bull. Entomol. Res. 16: 95-97.